UNIVERSITI PUTRA MALAYSIA

GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES OF WATER STRESSED FIG (Ficus carica L.) PLANT INFLUENCED BY ANTITRANSPIRANT-BRASSINOLIDE AND CARBON DIOXIDE ENRICHMENT

ZULIAS MARDINATA ZULKARNAINI

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By

ZULIAS MARDINATA ZULKARNAINI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor Philosophy

June 2020

DEDICATION

For my lovely wife Mellisa who believed in me, without you there would be no excuses for me to stand still and work hard to achieve my dreams. And for my mother Asnah, for all of your sacrifices and hardships in caring and teaching me as your son, you have raised me excellently. For my mother and father in law, my heartfelt gratitude for all love, encouragement, and support through the years of my quest for knowledge. May this achievement shall be our stepping stone towards living our dreams and ambitions...

The vegetation of a good land comes forth (easily) by the Permission of its Lord; and that which is bad brings forth nothing but (a little) with difficulty. Thus do We explain variously the Ayât (proofs, evidences, verses, lessons, signs, revelations, etc.) for a people who give thanks".

"[Al-A'râf 7 : 58]"

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES OF WATER STRESSED FIG (*Ficus carica* L.) PLANT INFLUENCED BY ANTI-TRANSPIRANT-BRASSINOLIDE AND CARBON DIOXIDE ENRICHMENT

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Chair : Assoc. Prof. Siti Zaharah Sakimin, PhD
Faculty : Agriculture

Fig *(Ficus carica* L.*)* belongs to the Moraceae family. It was a bush or small tree, moderate in size, deciduous with broad, ovate, 3 to 5-lobed leaves, contain copious milky latex and it was a new plant introduced in Malaysia. Brassinolide (BL) was a plant hormone which had biological effects on plant growth and development. While anti-transpirant containing magnesium (Mg) and calcium (Ca) as a main component was developed to increase photosynthesis and plant growth. Water stress (WS) adversely impacts many aspects of the physiology of plants, especially photosynthetic capacity. If the stress was prolonged, plant growth and productivity were severely diminished. In some other conditions, plants with high carbon dioxide (CO_2) concentration grew better than plants grown under ambient air conditions. The response of plant growth to CO_2 enrichment depends on the level of concentration, duration of CO_2 enrichment, nutrient availability, temperature, irradiance, water, and varieties. Little information on exogenous application of BL, anti-transpirant, elevated CO_2, and combination of them with WS on growth, physiological changes, and biochemical responses of fig plants. Thus, the aim of this study was (i) to investigate the effect of different concentration of exogenous application of BL on growth and physiological changes of fig var. Improved Brown Turkey (IBT) and Masui Dauphine (MD), (ii) to determine the effect anti-transpirant on growth and physiological changes under optimized BL concentration and best respond fig variety, (iii) to study the possible role of exogenously applied anti-transpirant in alleviating the detrimental effects of drought in fig under optimization of BL grown under a greenhouse and (iv) to study the effect short-term elevated CO_2 and water stress on biochemical responses and leaf gas exchange of fig under optimized BL concentration.

Fig plant was propagated using cuttings were transferred into mixed soil 3:2:1 (3 topsoil: 2 organic matters:1 sand). In Exp. 1, two different fig varieties (V) (IBT and MD) were sprayed into four levels (0, 50, 100, and 200 ml L^{-1}) of BL concentration. Different varieties of fig were considered as a main plot and BL concentrations (B) as a subplot. The experiment was arranged as a Split Plot Randomized Complete Block Design (SRCBD) with 4 replications (3 plants/rep). In Exp. 2, the plants were treated with four different concentrations (0, 2, 2.5, and 3 kg ha^{-1}) of anti-transpirant rate under optimized BL concentration using the best response of fig variety from exp. 1. The experiment was laid out as a Randomized Complete Block Design (RCBD) factorial with 3 replications (3 plants/rep). In Exp. 3, the plants were subjected to two WS levels: well-watered (WW) and water-stressed (WS). WS was defined at 100% and 25% water holding capacity, respectively. The best respond fig variety was selected and exogenously applied with optimum BL and anti-transpirant rate. The experiment was arranged as RCBD factorial with 3 replications (4 plants/rep). In Exp. 4, the plant was exogenously applied with optimum BL and subjected to two WS levels as similar as in Exp 3. The plant was placed under two different greenhouses conditions (elevated with 800 ppm CO_2 and without CO_2 elevated). Different greenhouses conditions were considered as main fixed effects and WS as a random effect. The experiment was arranged as Nested Design with 4 replications (4 plants/rep). Biochemical processes ([proline, malondialdehyde (MDA), protein, soluble sugar content (SSC), peroxidase (POD) and catalase (CAT) enzyme activities and starch]) and leaf gas exchanges [photosynthesis rate (A), stomatal conductance (Gs), transpiration rate (E), total chlorophyll content (T-Chl), relative chlorophyll content (CC), intercellular CO_2 (Ci), vapour pressure deficit (VPD), water use efficiency (WUE) and intrinsic-WUE] data were collected at monthly basis. All the data obtained were analyzed using Statistic Analysis System (SAS) version 9.4. A significant difference in mean values was determined and analyzed using two-way ANOVA and the mean differences were compared using the Least Significant Different Test (LSD) at 5% and 1% level of significance.

In exp. 1, the growth and physiological changes of the fig plants were affected by different application rates of BL and the cultivars. Total leaf area (TLA), specific leaf area (SLA), and shoot-to-root-ratio (S:R) increased with increasing concentrations of BL up to 100 ml L^{-1}, followed by a declining trend, whereas net assimilation rate (NAR) fluctuated throughout for of study. In the 1st month after treatment (MAT), increasing the BL concentration from 50 to 100 ml L^{-1} caused an increase in the NAR when compared to control but there was a decrease when BL concentration was 200 ml L^{-1}. At the 2nd MAT, by increasing the BL concentration from 50 to 200 ml L^{-1} had decreased the NAR. Application of BL had some effect on plant height (PH), TLA, total dry biomass (TDB), SLA, and NAR but it was not significant on the S:R. Among the varieties, IBT showed higher growth than MD at every five-weekly and monthly observation. There was a significant interaction between the BL and the variety for TLA, SLA, S:R, and NAR parameters. Additionally, only S:R parameter showed a significant effect of interaction between the BL and cultivar at 1% level of significance. Interaction between BL concentrations and fig variety was significant only at 5%. Like morphological parameters, physiological traits such as A, E, and CC have shown some differences with BL application, but the differences were not consistent and most of the changes happened only in the first or second month of observation. As levels of BL increased PH, TLA, TDB, and NAR parameters also linearly improved at 28%, 25%, 6% and 66%, respectively, higher than recorded for the control treatment.

In Exp. 2, as morphological parameters, physiological traits such as A, Es, Gs, WUE, Ci, and CC have shown some differences with BL application, but the differences were not consistent and most of the changes happened only in 1st or 4th MAT. Both the anti-transpirant and BL treatments were effective in the physiological responses of fig. BL treatments (control and 200 ml L^{-1}) were significant only at parameter chlorophyll fluorescence. Anti-transpirant concentration at 2 kg ha^{-1} and BL concentration at 200 ml L^{-1} showed higher physiological responses than the other concentrations at monthly observation. The growth stimulation was more pronounced on above-ground biomass than below-ground biomass, showing a high S:R. The increase in growth and physiology in this study might have been due to increased carboxylation rate after using the BL treatment, which enhanced carbon assimilation, channeling it to stimulate an increase in PH, TLA, and TDB.

In Exp. 3, drought substantially reduced the water status on Relative Leaf Water Content (RLWC), photosynthetic pigments, and leaf gas exchange. Moreover, substantially increased in biochemical responses attributes to proline content, MDA, SSC, POD, CAT but decreased on starch and protein content. However, the exogenous application of anti-transpirant remarkably improved the gas exchange and photosynthetic pigments both under drought and WW conditions. The results indicate that the application of anti-transpirant can ameliorate the effects of WW and enhance drought resistance of fig by adjusting water loss using stomatal control. Magnesium carbonate ($MgCO_3$) was considered to be an anti-transpirant that closes stomata and thus affects metabolic processes in leaf tissues. The anti-transpirant-induced increase in photosynthesis could be due to improvements in leaf water balance as indicated by increased water potential underwater-deficit and improved CC. Anti-transpirant application substantially enhanced the activities of enzymatic antioxidants. Furthermore, CAT activity was substantially enhanced later. This regulation of enzymatic antioxidants seems the result of anti-transpirant-induced regulation of transcription and translation, which led to the improvement in the level of SSC and enzymatic antioxidants and increment in MDA and proline content.

In Exp. 4, water deficiency specifically degraded the A, Gs, E, VPD, and WUE but increased Ci and int-WUE. Furthermore, a substantial increase in biochemical responses attributes to CC, proline content, MDA, SSC, POD, CAT but decreased on starch, protein content, T-Chl, and F_v/F_m. Nevertheless, elevated CO_2 concurrently increased the gas exchange and CCs both under drought and WW conditions. Underwater insufficiency, enriched CO_2 conditions boosted in physiological and metabolic activities were interceded through improved protein synthesis enabling maintenance of tissue water potential and activities of antioxidant enzymes reduction the lipid peroxidation. Differences in the short-term response to CO_2 enrichment may be also related to differences in the sink-source status of the whole plant depending on the developmental stages. Elevated CO_2 directly and indirectly, affects many plant physiological processes and biochemical accumulation in many plants and the identification of the key adaptive mechanisms to drought stress was essential to enhance the drought resistance of plants. Application of BL, anti-transpirant, and short-term elevated CO_2 increased growth, physiological changes, and biochemical responses of fig. However, the WS condition degraded the physiological processes of fig and triggered enzyme activities more active. Whereas, BL and anti-transpirant applications can ameliorate the effects of WS and enhance drought resistance of fig by adjusting water losses using stomatal control.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PROSES PERTUMBUHAN, FISIKOLOGI DAN BIOKEMIK TANAMAN TIN *(Ficus carica* L.*)* STRESS AIR SETELAH DIRAWAT DENGAN ANTI-TRANSPIRASI-BRASSINOLIDE DAN PENINGKATAN KARBON DIOKSIDA

Oleh

ZULIAS MARDINATA ZULKARNAINI

Jun 2020

Pengerusi : Prof. Madya. Siti Zaharah Sakimin, PhD
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Pokok tin *(Ficus carica* L.*)* tergolong dalam keluarga Moraceae. Ia adalah pokok belukar atau kecil, bersaiz moderat, daun daun yang lebar, ovate, 3 hingga 5 lobed, mengandungi getah yang banyak dan ia merupakan tanaman baru yang diperkenalkan di Malaysia. Brassinolide (BL) adalah hormon tumbuhan yang mempunyai kesan biologi terhadap pertumbuhan tumbuhan dan pembangunan. Anti-transpirant yang mengandungi Magnesium (Mg) dan Kalsium (Ca) sebagai komponen utama telah dibangunkan untuk meningkatkan pertumbuhan fotosintesis dan pertumbuhan pokok. Ketegasan air (WS) menjejaskan banyak aspek fisiologi tumbuhan, terutamanya kapasiti fotosintesis. Sekiranya ketegasan itu berpanjangan, pertumbuhan tumbuhan, dan produktiviti akan berkurangan. Dalam keadaan yang lain, tumbuhan dengan kepekatan CO_2 yang tinggi tumbuh lebih baik daripada tumbuh-tumbuhan yang ditanam di bawah keadaan udara ambien. Kesan terhadap pertumbuhan tumbuhan yang diberikan CO_2 bergantung kepada tahap kepekatan, tempoh pemberian CO_2, ketersediaan nutrien, suhu, air dan varieti. Terdapat maklumat yang terhad mengenai aplikasi BL secara luaran, anti-transpirant, pemberian CO_2 dan gabungannya dengan WS pada pertumbuhan, proses fisiologi dan tindak balas biokimia pada pokok tin. Oleh itu, matlamat kajian ini adalah (i) untuk mengkaji kesan pemberian BL kepekatan berbeza terhadap pertumbuhan dan proses fisiologi buah tin variti Improved Brown Turkey (IBT) dan Masui Dauphine (MD), (ii) untuk menentukan kesan anti-transpirant terhadap pertumbuhan dan perubahan fisiologi di bawah kepekatan BL yang dioptimumkan dan pelbagai tindakbalas varieti yang terbaik, (iii) untuk mengkaji kemungkinan peranan anti- transpirant dalam mengurangkan kesan buruk kemarau di bawah pengoptimuman BL yang ditanam di didalam rumah hijau dan (iv) untuk mengkaji kesan aplikasi CO_2 jangka pendek dan ketegasan air pada tindak balas biokimia dan pertukaran gas daun tin di bawah pengoptimuman kepekatan BL. Tanaman tin yang telah dibiakkan menggunakan keratan batang dipindahkan ke campuran tanah dengan nisbah 3:2:1 (3 tanah atas : 2 bahan organik : 1 pasir).

Dalam eksp. 1, dua varieti (V) pokok tin berbeza (IBT dan MD) telah disembur kepada empat tahap (0, 50, 100 dan 200 ml L^{-1}) BL. Pelbagai varieti tin yang berbeza dianggap sebagai plot utama dan kepekatan BL (B) sebagai sub plot. Eksperimen ini disusun sebagai split plot rawak lengkap (SRCBD) dengan 4 replika (3 tanaman/rep). Dalam eksp. 2, tumbuhan dirawat dengan empat kepekatan anti-transpirasi yang berbeza (0, 2, 2.5 dan 3 kg ha^{-1}) di bawah kepekatan BL yang dioptimumkan dengan menggunakan varieti fig yang memberikan tindak balas yang terbaik dari eksp. 1. Eksperimen ini dibentangkan sebagai Randomized Complete Block Design (RCBD) faktorial dengan 3 ulangan (3 tanaman/rep). Dalam eksp. 3, tumbuhan telah didedahkan kepada dua tahap WS: air yang disiram dengan baik (WW) dan tegasan air (WS). WS ditakrifkan pada kapasiti pegangan air sebanyak 100% dan 25% masing-masing. Pelbagai jenis tindakbalas terbaik dipilih dan aplikasikan secara semburan luaran kepada pokok dengan kadar BL dan anti-transpirasi optimum. Eksperimen ini disusun sebagai faktorial RCBD dengan 3 ulangan (4 tanaman/rep). Dalam eksp. 4, pokok fig dirawat secara semburan luaran pada permukaan daun dengan BL kadar optimum dan tertakluk kepada dua tahap WS seperti yang serupa dengan eksp. 3. Pokok yang menerima rawatan diletakkan di bawah dua keadaan rumah hijau yang berbeza (800 ppm CO_2 dan tanpa peningkatan kadar CO_2). Kondisi rumah hijau yang berbeza dianggap sebagai kesan tetap utama dan WS sebagai kesan rawak. Eksperimen ini disusun sebagai Reka Bentuk Bersarang dengan 4 ulangan (4 tanaman/rep). Tindak balas biokimia [proline, malondialdehyde (MDA), protein, kandungan gula larut (SSC), peroksida (POD) dan aktiviti enzim catalase (CAT) dan kanji] dan pertukaran gas daun [kadar fotosintesis (A), konduktiviti stomata (Gs), kadar transpirasi (E), total kandungan klorofil (T-Chl), kandungan klorofil relatif (CC), CO_2 antara sel (Ci), deficit tekanan wap (VPD), kecekapan penggunaan air (WUE) dan intrinsik-WUE] data dikumpulkan setiap bulan. Semua data yang diperoleh dianalisis menggunakan Sistem Analisis Statistik (SAS) versi 9.4. Perbezaan yang signifikan dalam nilai min ditentukan dan dianalisis dengan menggunakan ANOVA dua hala dan perbezaan min dibandingkan dengan Ujian Berbeza yang Rendah (LSD) pada tahap signifikan 5% dan 1%.

Dalam eksp. 1, pertumbuhan dan proses fisiologi pokok tin dipengaruhi oleh kadar aplikasi BL dan kultivar yang berlainan. Total leaf area (TLA), specific leaf area (SLA) dan nisbah shoot-to-root (S:R) meningkat dengan peningkatan kepekatan BL hingga 100 ml L^{-1}, diikuti oleh bentuk penurunan, manakala kadar assimilasi bersih (NAR) turun naik sepanjang tempoh kajian. Pada bulan pertama selepas rawatan (MAT), meningkatkan kepekatan BL dari 50 hingga 100 ml L^{-1} menyebabkan kenaikan NAR jika dibandingkan dengan kawalan tetapi terdapat penurunan apabila kepekatan BL adalah 200 ml L^{-1}. Pada MAT 2, dengan meningkatkan kepekatan BL dari 50 hingga 200 ml L^{-1} telah menurunkan NAR. Penggunaan BL mempunyai kesan ke atas ketinggian tumbuhan (PH), TLA, jumlah biomass kering (TDB), SLA dan NAR tetapi tidak signifikan ke atas S:R. Di antara varieti ini, IBT menunjukkan pertumbuhan yang lebih tinggi berbanding MD pada setiap pemerhatian lima minggu dan bulanan. Terdapat interaksi yang signifikan antara BL dan varieti fig bagi parameter TLA, SLA, S:R dan NAR. Tambahan pula, hanya parameter S:R yang menunjukkan kesan interaksi antara BL dan varieti pada tahap signifikan 1%. Interaksi antara kepelbagaian BL dan pelbagai varieti fig adalah signifikan hanya pada tahap 5%. Seperti parameter morfologi, ciri-ciri fisiologi seperti A, E, dan CC telah menunjukkan beberapa perbezaan dengan aplikasi BL, namun perbezaannya tidak konsisten dan kebanyakan perubahan berlaku hanya pada bulan pertama atau kedua pemerhatian. Oleh kerana peningkatan paras BL parameter PH, TLA, TDB dan NAR juga meningkat secara linear pada 28%, 25%, 6% dan 66% masing-masing, lebih tinggi daripada yang direkodkan untuk rawatan kawalan.

Dalam eksp. 2, seperti parameter morfologi, ciri-ciri fisiologi seperti A, Es, Gs, WUE, Ci dan CC telah menunjukkan beberapa perbezaan dengan aplikasi BL, namun perbezaannya tidak konsisten dan kebanyakan perubahan berlaku hanya pada MAT 1 atau 4. Kedua-dua rawatan anti-transpirant dan BL berkesan pada tindak balas fisiologi pokok fig. Rawatan BL (kawalan dan 200 ml L^{-1}) adalah signifikan hanya pada parameter fluroscen klorofil. Kepekatan anti-transpirant pada 2 kg ha^{-1} dan kepekatan BL pada 200 ml L^{-1} menunjukkan tindak balas fisiologi yang lebih tinggi daripada kepekatan lain pada pemerhatian bulanan. Rangsangan pertumbuhan biomas lebih ketara di atas tanah dibandingkan bawah tanah, menunjukkan S:R tinggi. Peningkatan pertumbuhan dan fisiologi dalam kajian ini mungkin disebabkan peningkatan kadar carboxylation selepas menggunakan rawatan BL, yang telah meningkatkan kadar asimilasi karbon dan menyalurkannya untuk merangsang peningkatan PH, TLA dan TDB.

Dalam eksp. 3, kemarau secara ketara mengurangkan status air pada kandungan air relatif daun (RLWC), pigmen fotosintetik dan pertukaran gas daun. Lebih-lebih lagi, tindak balas biokimia secara berterusan attribute kepada peningkatan kandungan proline, MDA, SSC, POD, CAT tetapi menurun pada kandungan kanji dan protein. Walau bagaimanapun, penggunaan anti-transpirasi secara luaran mengubah dengan baik pertukaran gas dan pigmen fotosintetik kedua-duanya di bawah keadaan kemarau dan air yang mencukupi. Keputusan menunjukkan bahawa penggunaan anti-transpirasi dapat memperbaiki kesan tekanan air dan meningkatkan ketahanan kemarau pokok fig melalui menyesuaikan kehilangan air menggunakan kawalan stomatal. Magnesium carbonate ($MgCO_3$) dianggap sebagai anti-transpirasi yang menutup stomata dan seterusnya mempengaruhi proses metabolik dalam tisu daun. Anti transpirasi menggalakkan peningkatan fotosintesis yang disebabkan oleh peningkatan keseimbangan air daun seperti yang ditunjukkan oleh peningkatan potensi air di bawah air dan CC yang bertambah baik. Permohonan anti-transpirasi meningkatkan aktiviti antioksidan enzim. Selain itu, aktiviti CAT juga dipertingkatkan kemudian. Peraturan antioksidan enzim ini seolah-olah hasil daripada pengawasan dan terjemahan yang disebabkan oleh anti-transpirant yang menyebabkan peningkatan tahap SSC dan antioksidan enzimatik dan peningkatan MDA serta kandungan proline.

Dalam Eksp. 4, kekurangan air secara khusus merendahkan A, Gs, kadar transpirasi, defisit tekanan wap dan kecekapan penggunaan air (WUE) tetapi peningkatan CO_2 antar sel dan WUE intrinsik. Tambahan pula, tindak balas biokimia yang meningkat secara berterusan terhadap kandungan klorofil relatif, kandungan proline, MDA, SSC, POD, CAT tetapi menurun pada kanji, kandungan protein, jumlah kandungan klorofil dan pendarfluor klorofil. Walau bagaimanapun, peningkatan CO_2 serentak meningkatkan pertukaran gas dan kandungan klorofil di bawah keadaan kemarau dan air bersih. Di bawah kekurangan air, keadaan CO_2 yang dinaikkan dalam aktiviti fisiologi dan metabolik telah diselaraskan melalui sintesis protein yang lebih baik yang membolehkan penyelenggaraan potensi air tisu dan aktiviti enzim antioksidan mengurangkan peroxidation lipid. Perbezaan dalam tindak balas jangka pendek terhadap pengayaan CO_2 mungkin juga berkaitan dengan perbezaan status tenggelam-sumber seluruh tumbuhan bergantung kepada peringkat perkembangan. Peningkatan CO_2 secara langsung dan tidak langsung, memberi kesan kepada banyak proses fisiologi tumbuhan dan pengumpulan biokimia di banyak tumbuhan dan pengenalpastian mekanisme penyesuaian utama kepada tekanan kemarau adalah penting untuk meningkatkan ketahanan tumbuhan tumbuhan. Penggunaan BL, anti-transpirant dan peningkatan jangka pendek CO_2 meningkat, perubahan fisiologi dan tindak balas biokimia daripada pokok tin. Walau bagaimanapun, keadaan tegasan air merendahkan tindak balas fisiologi rajah dan aktiviti enzim yang dicetuskan lebih aktif. Sedangkan aplikasi BL dan anti-transpirasi dapat memperbaiki kesan tegasan air dan meningkatkan kerintangan kemarau pokok fig dengan menyesuaikan kehilangan air pada daun menggunakan kawalan stomata.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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TABLE OF CONTENTS

UNDER OPTIMIZATION OF BRASSINOLIDE

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
*	significant at 0.05 probability level
**	significant at 0.01 probability level
#	number
$\mu mol\ m^{-2}s^{-1}$	micromol per meter square per second
$\mu mol\ mol^{-1}$	micromol carbon dioxide per mole air
^0C	degree-Celcius
L	Liter
A	net photosynthesis
A_{647}	Absorbance at 647 nm
ANOVA	analysis of variance
BL	Brassinolide
BRs	Brassinosteroids
BSA	Bovine serum albumin
$C_{28}H_{48}O_6$	2,3,22,23-Tetrahydroxy-β-homo-7-oxaergostan-6-one / Brassinolide
C_3	carbon 3 species
C_4	carbon 4 species
$CaCO_3$	Calcium Carbonate
CAM	crassulacean Acid Metabolism
CAT	catalase
CC	Chlorophyll Content
CGH	Control greenhouse (ambient CO_2 in a greenhouse)
Chl	chlorophyll
C_i	intercellular carbon dioxide concentration
cm^2	centimeter square
CO_2	carbon dioxide
C.V	coefficient of variation
d	day
DW	Dry weight
E	transpiration
EDTA	Ethylene diamine tetraacetic acid

e.g	example
FC	field capacity
F_m	maximal fluorescence
F_o	minimal fluorescence
F_v	variable fluorescence
f_v/f_m	The maximum quantum efficiency of PSII system
FW	Fresh weight
g	gram
g_s	stomata conductance
ha	hectares
H_2O	water
H_2O_2	Hydrogen peroxide
H_2SO_4	hydrogen sulphate
HCl	hydrochloric acid
hrs	hours
i.e	that is
IBT	Improved brown turkey
IPCC	Intergovernmental Panel On Climate change
lb	pound
$LSD_{0.05}$	least significance difference at 5% level
LRWC	Leaf relative water content
m	metre
MAT	Month After Treatment
MD	Masui dauphine
$MgCO_3$	Magnesium Carbonate
ml	Mili litre
mm	milimeter
MDA	malondialdehyde
mol $m^{-2}s^{-1}$	mole per meter square per second
MPC	Multi Purpose Cultivation
ms^{-1}	meter per second
n	number of samples
nm	nano meter

n.s	not –significant
NAR	net assimilation rate
P	probability
PGR	Plant growth regulator
POD	peroxidase
ppm	Part per million
PSII	photosystem II
PVP	polyvinylpyrolidone
RCBD	randomized complete block design
RGR	relative growth rate
RH	relative humidity
rpm	Rotary per minute
s	second
SE	Stem extension
SLA	specific leaf area
SPAD	soil plant analytical development
SRCBD	splitplot randomized complete block design
S/R	shoot to root ratio
SGH	Elevated CO_2 in the greenhouse
SRI	System of rice intensification
SSC	Soluble sugar content
t	time
TBA	thiobarbituric acid
TDB	Total Dry Biomass
T-Chl	Total Chlorophyll content
Temp.	temperature
TLA	Total Leaf Area
U	unit
UV	Ultraviolet
VPD	vapor pressure deficit
Var.	variety
W_0	Well watered
W_1	Drought stress
WUE	water use efficiency

WAT	weeks after the start of treatment
WS	Water stress
WW	wet weight

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Fig *(Ficus carica* L.*)* belongs to the Moraceae family. It is bush or small trees, moderate in size, deciduous with broad, ovate, 3 to 5-lobed leaves, contain copious milky latex and it is a new plant introduced in Malaysia. The fig fruit is a highly perishable climacteric fruit and the oldest species of the fruit tree having been cultivated by humans for over 5000 years (Owino *et al.*, 2006). The common fig *(Ficus carica* L.*)* is a tree indigenous to southwest Asia and the eastern Mediterranean region; belong to the family Moraceae (Duenas *et al.*, 2008). Figs are usually cultivated especially in warm, and dry climates.

The common fig grows wild in dry and sunny areas, with deep and fresh soil; also in rocky areas, from sea level to 1,700 meters. It prefers light and medium soils, requires well-drained soil, and can grow in nutritionally poor soil. The size, shape, colour of the skin, and pulp quality are markedly affected by climate. But quality figs are produced in the region with a dry climate especially at the time of fruit development and maturity. High humidity coupled with the low temperature usually results in fruit splitting and low fruit quality (Kislev et al., 2006).

The world's largest producer of dry as well as fresh figs is Turkey. Turkey produced 262 m^3 tons of Sar Lop, Bursa Black, and Yereven varieties or about 25% of the world's annual production. Egypt is the largest fig producing country in the Middle East, with 203 m^3 tons produced annually. Iran has an output of 75 m^3 tons, and Syria produces 44 m^3 tons each year. North Africa is also a major player in the fig industry, with Algeria, Tunisia, and Morocco producing a combined total of roughly 175 m^3 tons annually. The United States ranks eighth in global fig production, producing 43 m3 tons per year (Oberheu, 2018).

There are many benefits of figs for health. Fig helps to get rid of constipation, to help support and strengthen our bones, for losing weight, help digestion, cancer prevention, reduce hypertension, for a strong immune system, to treat sexual weakness as well, to cure diabetes, as skin cleaner to prevent cure acne, and a natural antioxidant (Fraser, 2014).

Many farmers are using brassinolide to increase their productivity. Brassinolide (BL) is a relatively new class of plant hormones showing a wide occurrence in the plant kingdom with unique biological effects on growth and development (Khripach *et al.*, 2000; Sun *et al.,* 2010). BL stimulates metabolic processes such as photosynthesis (Zhang *et al.,* 2008), nucleic acid, and protein synthesis (Bajguz, 2000). It also increases ATP*ase* activity in maize *(Zea mays* L.*)* roots, dark CO fixation, the activities of phosphoenol-pyruvate carboxylase (PEP*case*), and ribulose-1, 5-bisphosphate carboxylase (RuBP*case*), and soluble protein concentration in wheat leaf (Zhang et al., 2007).

Commonly used by greenhouses, anti-transpirant was a process previously thought of as impractical in open land cultivation. Some other anti-transpirant containing magnesium (Mg) and calcium (Ca) as a main component to increase photosynthesis and plant growth. It's a water-soluble compound composed of finely ground minerals that are sprayed onto a plant's leaf surface where it enters the leaf and discharges CO_2. The effects are increased plant growth, better yields, a reduction in water usage, and a more resilient plant. Anti-transpirant enters the plant via the stomata as a fine mist onto the leaf surface, when it has been sprayed and the minerals inside the plant discharge CO_2, It will increase glucose and proteins production and in turn, boost the quantity of oxygen released into the environment (Hartl and Stassen, 2007).

Water stress (WS) adversely impacts many aspects of the physiology of plants, especially photosynthetic capacity. If the stress was prolonged, plant growth and productivity were severely diminished, or even lead to plant death. Beside affected various plant physiology, WS imposes biochemical limitations and adverse effects (Bouranis *et al.*, 2014; and Chen *et al.*, 2015). Cell growth is the process that is most affected by water deficit. Furthermore, under more severe drought conditions inhibition of cell division, inhibition of wall and protein synthesis, and accumulation of solutes (Taiz and Zeiger, 2002).

C_3 plants can respond positively to increases in the air's CO_2 concentration by exhibiting enhanced rates of photosynthesis and biomass accumulation, especially under conditions of water insufficiency, in contrast to the erroneous view that such plants will not benefit from earth's rising atmospheric CO_2 concentration (Lim, 2012).

Several studies have investigated the effect of brassinolide on water-stressed plants. Li *et al.* (2007) reported that application 0.4 mg/L brassinolide increased the survival, growth, and drought resistance of Robinia pseudoacacia seedlings under water stress. Similarly, Zhang *et al.* (2008) found that brassinolide significantly increased drought tolerance and minimize the yield loss of soybean caused by water deficits. Meanwhile, Aldasoro *et al.* (2018) resulted that the physiological plant responses to the application of the anti-transpirant both under well-watered and water-deficit conditions reduce the negative effects of drought in nodulated legumes. Del Amor *et al.* (2010) studied application anti-transpirant under enhancing CO_2 and water stress condition. They reported that the application of anti-transpirant increased on photosynthesis and water relations of pepper plants under different levels of CO_2 and water stress especially after 4 days of drought. However, the effects of exogenous application of BL, anti-transpirant, elevated CO_2, and combination of them with WS on growth, physiological changes, and biochemical processes have not been reported especially on fig plants.

1.2 Problem Statements

Information on anti-transpirant-brassinolide application exogenously under condition water stress is still lacking especially in the tropical zone and on fig plant. Studies about physiological and biochemical responses of fig under CO_2 enrichment techniques in the microclimate conditions are scarce as well. Consequently, farmers' productivity will decrease too. Hence, to benefit from the application it is pertinent to establish the CO_2 enrichment technique especially the protocol for difficult and slow-growing fruit plant species like fig should be established.

1.3 Hypothesis

1. Fig varieties alone and BL alone would increase the growth and physiological processes of fig.
2. BL and anti-transpirant interaction will optimized growth resulting from improved fig plant physiological and biochemical processes.
3. Exogenous application of anti-transpirant would alleviate the detrimental effects of drought in fig under optimization of BL evaluated in a greenhouse.
4. Elevated CO_2 and WS would influence on biochemical processes and leaf gas exchange of fig under BL optimization

1.4 General Objective

A project consists of four experiments was conducted to enhance growth and study about physiological and biochemical processes of stressed fig plant by the application of BL, anti-transpirant, along with microclimate CO_2 enriched conditions.

1.5 Specific Objectives

A project consisting of four major experiments hence, it has carried out with the overall objectives:

1) to investigate the growth and physiological processes of two fig cultivars;
2) to determine the effect anti-transpirant on growth and physiological processes under best respond fig variety and optimized BL application;
3) to study exogenous anti-transpirant and optimized brassinolide application in alleviating the detrimental effects of the drought of fig under greenhouse condition; and
4) to study the effect of short-term elevated CO_2 on biochemical processes and leaf gas exchange of water-stressed fig *(Ficus carica* L.*)* as influenced by optimized brassinolide application.

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PUBLICATIONS

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